Applying Biomarker Research

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Public health and environmental professionals have generally focused on monitoring the ambient environment to assess exposures to the public. To understand exposures and effects and predict onset or course of disease, it is also important to look inside the (human) organism.

Biomarkers—measurable internal indicators of changes in organisms at the molecular or cellular level—offer great potential to understand environmentally mediated disease and to improve the process of risk assessment. A valid biomarker could also be considered a key event linking a specific environmental exposure to a health outcome.

A molecular biomarkers paradigm has its origins in the National Research Council's (NRC) 1983 "Red Book," *Risk Assessment in the Federal Government* (*1*). In 1989, the NRC published monographs on *Biologic Markers in Pulmonary Toxicology* (*2*) and *Biologic Markers in Reproductive Toxicology* (*3*) in which biomarkers of exposure, effect, and susceptibility were discussed as they may relate to disease. In Figure 1, the exposure-to-disease paradigm has been drawn to emphasize the application of biomarkers in assessing dose, mode-of-action, and disease etiology. Biomarkers of susceptibility may influence the magnitude of each sequential element in the pathway. Biomarkers useful for disease prevention and intervention may appear anywhere along the pathway. Earlier markers (to the extent that they are measurable at low exposure or dose) have the greatest potential utility to avert disease; later markers are most closely related to the disease.

Over the last decade the biomarker model has resulted in considerable research enterprise and nourished and challenged the emerging field of molecular epidemiology. The National Institute of Environmental Health Sciences (NIEHS) and the U.S. Environmental Protection Agency (U.S. EPA), in both their intramural and extramural research programs, have targeted biomarkers for a greater role in human health risk assessment and have worked for their validation in the field and their extension into the clinical environment. At the same time, much of the biomarker research has remained confined to the laboratory, with the promise of successful applications to improve public health or mitigate disease largely unmet.

A biomarker should allow better measurements of exposure or earlier identification of health effects. Biomarkers can provide data needed for assessing progress in improving the Nation's health, such as the *Healthy People 2010* objectives (*4*). In summary, biomarkers may break open the black box between exposure and disease and show what individual factors make a difference.

Recognizing their substantial investments in intramural and extramural research on biomarkers, the NIEHS and the U.S. EPA held "Biomarkers: Taking Stock, An EPA/NIEHS In-House Workshop on Applying Biomarker Research" on 30–31 August 1999 in Chapel Hill, North Carolina. Approximately 90 participants explored biomarker research through presentations by invited plenary speakers, posters on individual research projects, and breakout discussion groups.

Participants focused on both scientific and organizational objectives. Scientifically, they sought to understand the state of the art and current applications of biomarkers of exposure, effect, and susceptibility; to discern research directions that are likely to make the promise of usable biomarkers a reality; and to explore the role of biomarkers in understanding environmentally-induced disease and in assessing human health risk. Organizational objectives were to increase communication among NIEHS and U.S. EPA staff, including those involved in clinical studies; to complement the ongoing environmental genome research at NIEHS and the planned NIEHS workshops on exposure assessment and environmental epidemiology; and to lay the groundwork for a possible extramural conference on biomarkers in spring 2001.

Workshop Discussion Themes

Biomarkers in risk assessment. There is a need to move toward biologically based risk assessments. Biologically based risk assessments will refine estimates of dose to relevant targets through the use of biomarkers of exposure. They will improve hazard characterization through the use of biomarkers of response or effect with mechanistic linkage to end points of concern. They will strengthen inferences regarding the shape of dose–response curves outside the range of observation and identify targets of opportunity for further study in potentially sensitive human populations. Biomarkers of exposure, effect, and susceptibility are intimately linked; conceptually it may be helpful to pull them apart but in reality they are integrated. Recognizing this linkage allows for directly tying biomarkers to testable hypotheses. This requires creative study design, most often drawing on researchers from several disciplines.

The U.S. EPA, in its *Proposed Guidelines for Carcinogen Risk Assessment* (*5*), and more recently the International Programme for Chemical Safety, with its generic "Framework for Risk Assessment" (*6*), have developed similar frameworks for bringing a greater variety of scientific information into risk assessment. The basic framework is applicable to all end points—cancer as well as noncancer.

In this framework the concepts of mode of action and key events play central roles. We must distinguish mode of action from mechanism of action. Mechanism of action is defined as the detailed molecular description of the events involved in the induction of cancer or other health end points. Mode of action links key events and sequential processes, starting with the interaction of an agent with a cell, through functional and anatomical changes, and resulting in cancer or other health end points. Consideration of mode of action raises three questions: How does the chemical produce its effect? Are there mechanistic data to support this hypothesis? Have other mechanistic hypotheses been considered and rejected?

To show that a postulated mode of action is operative, it is generally necessary to outline the sequence of events leading to the toxicologic or disease end point and to identify key events that can be measured, often through biomarkers. Then it is necessary to weigh the available information to determine whether the key events are on the pathway to causality. A key event is defined as an empirically observable precursor step that is a necessary element of the mode of action or is a marker

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This manuscript has been reviewed by the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Received 16 March 2000; accepted 22 May 2000.

PM3006509210

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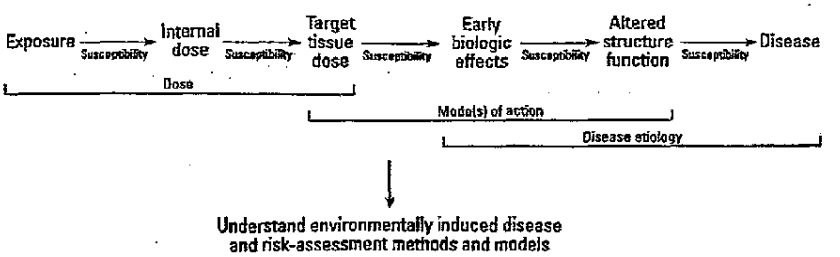

Figure 1. Applying biomarkers in assessing dose, mode of action, and disease etiology.

for such an element. Examples of key events include metabolism, receptor–ligand changes, increased cell growth and organ weight, hormone or other physiologic perturbations, hyperplasia, and cellular proliferation.

It is also important to understand the background for dose–response analyses. When we estimate risk from exposures we look at incremental exposures—the impact of a specific exposure on overall risk. Therefore, we must know something about the background level of the chemical to which the increment added, as well as the background of similar chemicals that operate by the same mode of action. Finally, we must know the background of disease so that we can understand where we are on the dose–response curve. This is an area where biomarkers can be applied quite well.

Clearly, this new framework will require substantial basic and applied research to provide the information needed for risk assessment. It also provided a useful construct for consideration of applications of biomarkers at the workshop.

Biomarkers of susceptibility. An individual's susceptibility to environmentally mediated disease may arise from genetic causes or from nongenetic factors such as age, disease state, diet, or dietary supplementation. Most of the discussion at the workshop centered on understanding genetic susceptibility.

Polymorphisms may be markers of susceptibility. The rapid advances of the Human Genome and Environmental Genome projects are generating a long list of genes and their variants (polymorphisms). Research is helping us to understand which genes are perturbed on the pathway to disease. Many of these genes are quite general in their function and broadly applicable to the assessment of susceptibility. Such genes or groups of genes will, for example, influence or control cell differentiation, apoptosis, cell cycle kinetics, or DNA repair. Receptor-mediated pathways involving alterations in signal transduction can influence a variety of health outcomes. There is a spectrum of human genetic variability such that a distribution of responses to a given exposure can sometimes be predicted.

New technologies such as microchip arrays allow researchers to explore patterns of gene expression. In the face of this burgeoning information and data being gathered in National Institutes of Health and other databases, the challenge for researchers interested in environmentally mediated disease or risk assessment is to understand the functionality of genetic polymorphisms and to relate this to disease. We may gain this understanding in humans, particularly by relating laboratory, clinical, and epidemiologic findings. To date, most genetic susceptibility studies have looked at cancers as an end point, although research on other diseases such as asthma is beginning to grow. As our understanding of functionality grows, so will our need for understanding of the ethical implications of our knowledge to individuals and society.

Biomarkers of exposure. Advancing the utility of biomarkers of exposure requires a multidisciplinary effort. Biomarkers of exposure may relate external exposure to internal dose, provide measurable events in dose–response assessment, and document exposures in epidemiologic studies and clinical evaluations.

Biomarkers that integrate exposures from different pathways or media, thereby integrating dose, are of great interest, particularly with regard to mixtures. Microarray technology has the potential to identify common patterns of gene expression thus potentially confirming exposures even to mixtures, but studies of functionality of protein products must also be carried out.

Biomarkers are of value in site-specific assessment, such as at Superfund sites. Clearly biomarkers have value and have been used in assessing acute exposures and acting as triggers for action, for example in responding to accidental mercury or methylparathion exposures. Lead in blood, plasma, or bone is an excellent biomarker of exposure and potentially of effects. Lead biomarkers also illustrate a challenge in understanding the value of information. If we measure lead in blood in a small population of children on or near a site and find high blood lead concentrations, there is clearly cause for concern and action. If, however, blood lead concentrations are not elevated compared to national statistical measures or a local control group, does this mean that the site presents no problem and there is no need to act? In this case questions of both representativeness of samples and population statistics become paramount.

The U.S. EPA National Human Exposure Assessment Survey (NHEXAS) (*7*) is one example of a large exposure-based experimental design on a community or regional level. We begin with blood, urine, and breath measurements and then return to look at what people had been exposed to by measuring the contents of their drinking water, food, and ambient air to see whether there are correlations. NHEXAS data are now being analyzed and should yield informative results.

We need to take a public health approach to demonstrate the relationship of biomarkers to human disease. Advances in molecular biology have improved the linkage of biomarkers of effects with biomarkers of exposure, making a top-down public health approach more feasible, facilitating attribution of causality, and providing guidance for decision-making and intervention.

Biomarkers of effect. Biomarkers of effect may be either early events on the direct pathway to disease or toxic end points or predictors of disease or toxicity outside the direct pathway, i.e., they covary with the toxic process or disease (Figure 2).

The challenge is to link biomarkers to human disease both through human and animal studies. Some approaches would begin with exposures or highly exposed populations and look for biomarkers associated with effects or disease end points. Others would look for early biomarkers of the disease itself and then work backward to look at populations that have exposures to various agents to see if the biomarker rises as their exposure rises. In either case establishing linkage between exposure and disease is critical.

For studying biomarkers in a human population, imaging technologies such as magnetic resonance or positron emission tomography are particularly interesting because they are noninvasive and measure change at a molecular scale.

Successful use of biomarkers of effect, and in some cases biomarkers of exposure, requires knowing what they mean in terms of health and disease. Most have yet to be validated for ascertainment of health or disease.

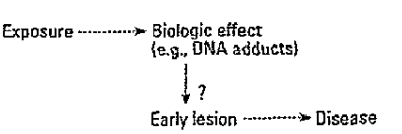

Figure 2. Biomarkers of effect: on the pathway to disease?

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Biomarkers in susceptibility evaluation and disease intervention. Development of biomarkers for environmental agents should be based on knowledge of metabolism, product formation, and general mechanisms of action. Validation requires parallel experimental and human studies.

Figure 3 illustrates a systematic approach for the validation and application of chemical-specific biomarkers to human studies (*8*). The two end points for this process are validated markers of exposure and markers of risk. The paths to the end points pass through hazard identification, developing reliable methodologies for measuring biomarkers, parallel studies in animals and exposed humans, and epidemiologic studies and clinical trials. The approach could be applied to noncancer end points as well to cancer.

This validation approach has been applied in exhaustive studies relating *a*) exposures to aflatoxin B_1, *b*) the etiology of human hepatocellular carcinoma (HCC), and *c*) intervention with oltipraz as a chemopreventive agent for HCC (*8*). Biomarkers included aflatoxin–albumin adducts in serum and aflatoxin–mercapturic acid excreted in urine.

Given the multistage process and long latency of cancer and other chronic human diseases, it is likely that relatively few individual chemical-specific biomarkers will prove to be validated risk markers. Rather, validated risk markers may turn out to be composites of chemical-specific biomarkers and other markers, each of which contributes in some quantifiable way to determining overall risk (*8*).

Biomarkers in epidemiologic studies. The most frequent applications of biomarkers in molecular epidemiology have been in the assessment of exposure (or dose) and susceptibility due to genetic and nongenetic factors.

A recent study of the developmental effects of fetal exposure to polycyclic aromatic hydrocarbons (PAHs) via ambient pollution illustrates the utility of molecular epidemiology in identifying potential etiologic factors in disease (*9*). The study was intended to generate hypotheses for further research in an area that has proven elusive. To complement and confirm environmental monitoring and questionnaire data, investigators used biomarkers to estimate the individual dose of toxicants to the fetus.

In the study, PAH–DNA adducts in leukocytes and plasma cotinine were the biomarkers measured in umbilical cord blood as dosimeters of transplacental PAHs and cigarette smoke, respectively. Cotinine, a nicotine metabolite, is uniquely associated with cigarette smoke and provides a means to ascertain the relative contribution of smoking to a parameter such as PAH–DNA adducts and any observed outcome.

Researchers have not yet realized a goal of molecular epidemiology to develop preclinical markers that are linked closely enough to disease that they are suitable for screening studies, preventative actions, and diagnosis. They have vividly demonstrated the complexity of the multiple factors that determine among those exposed who will get the disease and who will not. Batteries of biomarkers may be needed to characterize this complexity.

Historically much of the research in molecular epidemiology has been opportunistic, driven by what populations were available, what samples have been collected, what could be measured in an available sample; out of that one might develop an hypothesis to test. This was appropriate in the early stages of biomarker validation. Our understanding of the science supporting molecular epidemiology has now advanced so that we are prepared to undertake systematic approaches such as the validation model. This will allow us to distinguish a key event on a causal pathway from simply a statistical association.

Biomarkers in model development. Risk-assessment models often depend on interpretation at the level of the individual rather than the population. Generally, surrogate markers must be used to represent target tissues. The models facilitate interspecies extrapolation and investigation of the effects of lower doses/exposures. The models also facilitate the integration of epidemiologic and clinical information, *in vivo* and *in vitro* data, and structure–activity concepts.

There is a need for sharing data at the individual level. It is difficult to develop and validate risk assessment models based on the use of data from the literature that consist only of means and standard errors. Modelers need to know what is going on in a variety of situations at the individual human or animal level. This is important for both the scientists designing or conducting studies and the regulatory community trying to apply this information.

An intended use of the biomarker may be in the clinical setting, where the focus is on the individual and there is an opportunity to gather substantial detailed information. Frequently, however, the intended use is in the population setting where exposures are diverse and information about the exposure and the population of interest is limited. In the laboratory setting, where *in vitro* and animal studies are the norm, there is a substantial advantage in experimental control.

Factors that influence interindividual variation include physiology, exposure, environment, and genetics. The effects of glutathione transferase theta polymorphism on the risk estimates for dichloromethane in humans are an example of the influence of genetics (*10*).

Recommendations

Research emphases. Based on the discussions at the workshop, the planning committee identified the following scientific areas for emphasis in NIEHS and U.S. EPA programs:

- Conduct more prospective (epidemiologic) studies linking exposure to disease, especially noncancer diseases
- Apply new technologies (e.g., microchip arrays) to develop more incisive markers
- Make better use of animal models to develop and link biomarkers to disease in humans
- Use Bioinformatics to make sense of existing data (*12*)
- Continue efforts to discover genetic basis of risk factors
- Conduct phenotypic and functional studies of genetic polymorphisms
- Use genetic susceptibility information to evaluate risk distributed across populations
- Look for risk factors beyond genetic susceptibility, including age, nutrition, lifestyle, and sex
- Develop mechanistic information to link external exposure to internal dose, particularly for cumulative or aggregate exposures
- Increase emphasis on interdisciplinary approaches, including cross-training programs and research
- Use more biomarkers in exposure and health initiatives

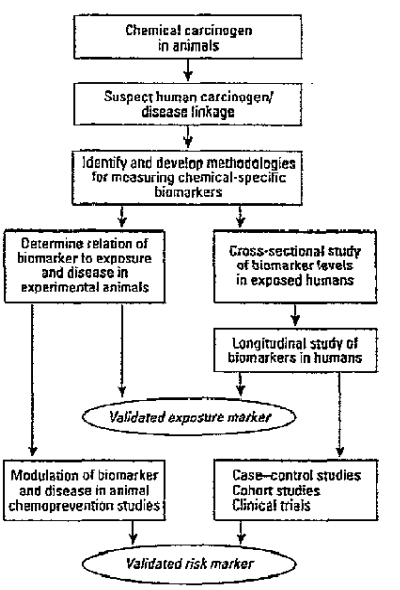

Figure 3. Model for validating chemical-specific biomarkers. Reproduced from Groopman and Kensler (*8*) by permission of Oxford University Press.

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- Apply biomarkers to site-specific risk assessment.

Enhancing research collaboration. Enhanced research collaboration in areas related to biomarkers is a subject of great interest within the federal government. Looking at exposure assessment alone, before the end of 2000:

- The NIEHS will publish its report and recommendations from the workshop on "The Role of Human Exposure Assessment in the Prevention of Environmental Disease" (*11*).
- The U.S. General Accounting Office will issue a report assessing *a*) the extent to which federal and state agencies collect or use human exposure data on potentially harmful chemicals, including data needed to identify at-risk populations and *b*) the main barriers that hinder further progress in such efforts.
- The Committee on Environment and Natural Resources of the President's Office of Science and Technology Policy will develop a strategy document for consideration as a fiscal year 2002 budget initiative. This initiative will be interagency and facilitate leveraging of resources across several agencies that have made exposure assessment a priority area.

Governmental initiatives in research collaboration will consider the results of all of these activities.

As their contribution to these considerations, participants at the biomarkers workshop developed specific ideas to enhance collaborations between the U.S. EPA and the NIEHS (and others) to promote innovative biomarkers research and application. Examples of these collaborations include:

- Supporting postdoctoral investigators who are employed on projects that involve investigators from both institutions
- Jointly planning intramural and extramural requests for applications/requests for proposals that will encourage the U.S. EPA and the NIEHS (and others) to collaborate on innovative biomarkers research
- Joining the exposure expertise of the U.S. EPA and the NIEHS with epidemiologic, toxicologic, and biomarkers expertise of both institutions
- Establishing joint NIEHS/U.S. EPA workshops, working groups, or seminars on topics of mutual concern, such as epidemiologic studies being planned or in progress, microarray technology, or genotyping methods
- Using the World-Wide Web creatively through the NIEHS and the U.S. EPA home pages or a separate Web site that could identify ongoing epidemiologic and toxicologic studies and seminars, or support interest groups, e-mail list servers, discussion groups, etc., to stimulate collaborations
- Looking for specific opportunities for collaboration in existing/developing research programs, e.g., NHEXAS (*7*) the National Cancer Institute/NIEHS/U.S. EPA Agricultural Health Study (*13*); the National Toxicology Program study design including animal/sample sharing (*14*); and site-specific risk assessment (opportunity to apply biomarkers in the field)
- Sharing resources, especially specialized equipment, between the two institutions.

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